

Attorney Docket No. 3817.14-1  
Customer No. 23308

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Inventor	: Tabassum Naqvi	Art Unit.	: 1641
Serial Number	: 10/689,122	Examiner	: HAQ, Shafiqul
Filed	: 10/20/2003		:
Title	: IP <sub>3</sub> Protein Binding Assay		:
Confirmation Number	: 4234	Customer Number	: 23308

<p align="center"><b>CERTIFICATE OF MAILING or FACSIMILE TRANSMISSION UNDER 37 C.F.R. § 1.8(a)(1), or ELECTRONIC FILING UNDER 37 C.F.R. §1.8(a)(1)(C)</b></p> <p>I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being</p> <p>_____ deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to Mail Stop _____, Commissioner for Patents, P.O. Box 1450, Alexandria Virginia, 22313-1450,</p> <p>_____ facsimile transmitted to the U.S. Patent and Trademark Office on the date shown below.</p> <p>_____ transmitted via the Office electronic filing system in accordance with §1.6(a)(4)</p> <p>Dated: _____ By: _____</p> <p>Printed Name: _____</p>
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**DECLARATION UNDER 37 CFR §1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

I, Edwin F. Ullman, Ph.D., hereby declare as follows:

1. I am a consultant for the assignee of the subject application. My c.v. accompanies this declaration. I was asked to review the reference cited in the Official Action, Hirata, et

Attorney Docket No. 3817.14-1  
Customer No. 23308

al., JBC (1990) 266, 8404, and provide my evaluation of the conclusions that may be reasonably drawn from the experimental work reported in the paper.

2. This paper describes the behavior of D-IP<sub>3</sub> and its 2-hydroxy ester derivatives, D- and L-206 and D- and L-209.

3. This paper shows the following:

Fig 2 shows the inhibition of IP<sub>3</sub>-5-phosphatase activity in erythrocyte ghosts by the above compounds. The activity was determined by measuring the decrease in [<sup>3</sup>H]IP<sub>3</sub>. Only D-IP<sub>3</sub> and L-209 produce a significant decrease in the rate. This shows that the 2-substituted D-IP<sub>3</sub> derivatives do not bind at the active site of this enzyme.

Fig 4 shows the inhibition of IP<sub>3</sub> kinase activity in rat brain cytosol by the above compounds. The activity was determined by measuring the increase in [<sup>3</sup>H]IP<sub>4</sub>. Only D-IP<sub>3</sub>, D-206 and D-209 were inhibitory. Whether the inhibition was actually due to binding of D-206 and D-209 to IP<sub>3</sub> kinase is questionable (see comments on Fig 5).

Fig 5 shows chromatograms of the mixtures obtained by reaction of each of the above compounds with [<sup>32</sup>P]ATP in the presence of rat brain cytosol. Both D-IP<sub>3</sub> and D-206 were converted to [<sup>32</sup>P]IP<sub>4</sub>. This suggests that D-206 was first hydrolyzed to IP<sub>3</sub> by esterases which was then phosphorylated by an IP<sub>3</sub> kinase. Because kinases are usually highly specific it is less likely that D-206 could have first been phosphorylated and the product then hydrolyzed to IP<sub>4</sub>. If the former is correct, the apparent Fig 4 conclusion that D-206 inhibits IP<sub>3</sub> kinase activity is not justified. It is more likely that the IP<sub>3</sub> formed from D-206 inhibited IP<sub>3</sub> kinase activity .

Fig 5 shows that D-209 was not converted to IP<sub>4</sub> or to any other unique ATP reaction product. It is therefore neither a substrate for the kinase or the esterases, and it is

Attorney Docket No. 3817.14-1  
Customer No. 23308

possible that it is a true inhibitor of IP<sub>3</sub> kinase as suggested by Fig 4. Although less likely, it is also possible that its IP<sub>3</sub> kinase inhibitory activity arises from the product of some other reaction product such as dephosphorylation (see following paragraph), and that it too is not a true IP<sub>3</sub> kinase inhibitor.

The Fig 5 results are further complicated by the appearance of a <sup>32</sup>P-labeled product (D- and L-isomers are not distinguishable) that elutes in 13 minutes and is common to all of the compounds. This requires that all four of the esters undergo hydrolysis, phosphorylation, and possibly dephosphorylation by enzymes that are present in rat brain cytosol. This observation further supports the likelihood that esterases play an important role in the kinase inhibition study.

Fig 6 appears to show that D-IP<sub>3</sub>, D-206 and D-209, but not L-206 and L-209, can compete with [<sup>3</sup>H]IP<sub>3</sub> for a binding site present in cerebellum microsomes. However this conclusion is not justified because of the likely possibility that the inhibition is due to esterase catalyzed hydrolysis of D-206 and D-209 to D-IP<sub>3</sub>.

4. To summarize:

Binding of D-IP<sub>3</sub> to IP<sub>3</sub>-5-phosphatase in erythrocyte ghosts is not inhibited by the 2-hydroxy esters, D-206 and D-209.

There is suggestive but non-rigorous evidence that D-209 binds to rat brain cytosol IP<sub>3</sub> kinase but there is no evidence that D-206 binds to this protein.

D- and L-206 and D- and L-209 or their phosphorylation or dephosphorylation products are substrates for rat brain cytosol esterases. There is no evidence as to whether one or more esterases are involved or the order of the steps leading to the common product.

D-206 and D-209 might compete with [<sup>3</sup>H]D-IP<sub>3</sub> for binding to a protein in cerebellum microsomes, but the foregoing suggests that it is more likely that esterases in the microsomes convert one or both of these compounds to D-IP<sub>3</sub> which competes with [<sup>3</sup>H]D-IP<sub>3</sub>.

5. Conclusion

Because the experiments were carried out with different tissue preparations, each containing complex mixtures of binding proteins, this paper provides few definitive conclusions. The data suggest but do not prove that a single 2-hydroxy ester, D-209, binds to a rat brain cytosol IP<sub>3</sub> kinase. Apparent binding of the other 2-hydroxy ester, D-206, is likely due in whole or in part to formation of D-IP<sub>3</sub> upon esterase catalyzed hydrolysis of D-206. Moreover neither of these compounds bind to the IP<sub>3</sub> binding protein, IP<sub>3</sub>-5-phosphatase, in erythrocyte ghosts, and the authors' conclusion that they bind to cerebellum microsomes proteins is unjustified. Accordingly there is no showing that 2-hydroxy D-IP<sub>3</sub> esters that bind to a particular IP<sub>3</sub> kinase binding site are able to bind other IP<sub>3</sub> kinases, and there is strong evidence that they cannot bind generally to any D-IP<sub>3</sub> binding protein.

6. Based upon these observations, it is my finding that one would have good reason to conclude from the paper that the 2-IP<sub>3</sub> derivatives would be unlikely to have affinity for the IP<sub>3</sub> receptor. Furthermore, in the subject application, the IP<sub>3</sub> receptor is not used for binding to the 2-derivative of IP<sub>3</sub>, but rather a truncated receptor is used that has a much higher binding affinity. Therefore, neither does the reference support the conclusion that the IP<sub>3</sub> receptor would be expected to have a specific affinity for the 2-derivative of IP<sub>3</sub>, nor does the subject application even use this protein. It would not have been obvious to expect that the 2-derivative of IP<sub>3</sub> would have a high affinity for the "sponge protein" derived from IP<sub>3</sub> receptor or for any other protein than possibly the IP<sub>3</sub> kinase from rat brain cytosol.

Attorney Docket No. 3817.14-1  
Customer No. 23308

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Date: Jan 21, 2008

By: Edwin F. Ullman  
Edwin F. Ullman, Ph. D.

Attachment: Curriculum Vitae

## **CURRICULUM VITAE**

**Edwin F. Ullman**  
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## **OCCUPATION**

Consultant for biotech and biotech investment companies

## **FIELDS OF EXPERTISE**

### SCIENCE AND TECHNOLOGY

Immunochemistry and immunoassay methods  
Nucleic acid chemistry and assay methods  
Analytical methods and devices in clinical and medicinal chemistry  
Diagnostics and high throughput screening  
Chemistry of stable radicals as spin labels  
Organic photochemistry including fluorescent dye labels  
Organic and photochemical reaction mechanisms.

### MANAGEMENT

R&D Direction  
Corporate Strategies  
Patenting Strategies  
Expert Witness for patent litigation

## **EMPLOYMENT**

Research Chemist, Lederle Laboratories, American Cyanamid Company  
Pearl River, New York, 1956-60

Group Leader, Central Research Division, American Cyanamid Company, Stamford,  
Connecticut, 1960-66

Scientific Director, Synvar Research Institute, Palo Alto, California, 1966-70

Vice President and Director of Research, Syva Company  
Palo Alto, California, 1970-95

Vice President and Director of Research, Behring Diagnostics Inc.  
San Jose, California, 1995-97

Attorney Docket No. 3817.14-1  
Customer No. 23308

Chief Scientific Officer, ThauMDx, LLC, 2001-2

Chief Scientific Officer for Genomics and Advisor (part time), DiscoverRx Corp.,  
2002-2005

Advisor (part time), Innovations Research, 2004-2005

### **EDUCATION**

Reed College, Portland, Oregon, BA., 1952

Harvard University, Cambridge, Massachusetts, MS., 1954 Organic chemistry

Harvard University, Cambridge, Massachusetts, Ph.D., 1956 Organic chemistry

### **AWARDS AND HONORARY POSITIONS**

Phi Beta Kappa, 1952

National Science Foundation Fellow, 1952-3

Harvard Teaching Fellow, 1953-4

US Rubber Company Fellow, 1954-5

Clinical Ligand Assay Society, Mallinckrodt Award, 1981

Canadian Clinical Chemistry Society, MSD Health Group Award, 1982

New York Metropolitan Section of American Association for Clinical Chemistry  
Van Slyke Award, 1984

American Association for the Advancement of Science Fellow, 1987

Peninsula Patent Law Association Inventor-of-the-year Award, 1987

Northern California American Association of Clinical Chemists

Outstanding Contributions to Clinical Chemistry Award, 1991

American Association for Clinical Chemistry, Award for Outstanding Contributions to  
Clinical Chemistry in a Selected Area of Research, 1997

The American Association for Clinical Chemistry and Dade Behring co-sponsor the  
Edwin F. Ullman Award given annually since 1998 by the American Association  
for Clinical Chemistry.

### **EDITORIAL AND EDUCATIONAL ADVISORY BOARDS**

Journal of Organic Chemistry Editorial Advisory Board, 1969-74

Journal of Immunoassay Editorial Board, 1979-

Journal of Clinical Laboratory Analysis Editorial Board, 1985-87

San Francisco State University Science & Engineering Advisory Board , 1994-96

Journal of the Clinical Ligand Assay Society, Editorial Board, 1999-01

Attorney Docket No. 3817.14-1  
Customer No. 23308

**MEMBERSHIPS**

American Chemical Society  
American Association of the Advancement of Science  
American Association of Clinical Chemists

**PUBLICATIONS AND PATENTS**

Over 110 scientific publications and 230 issued US patents.